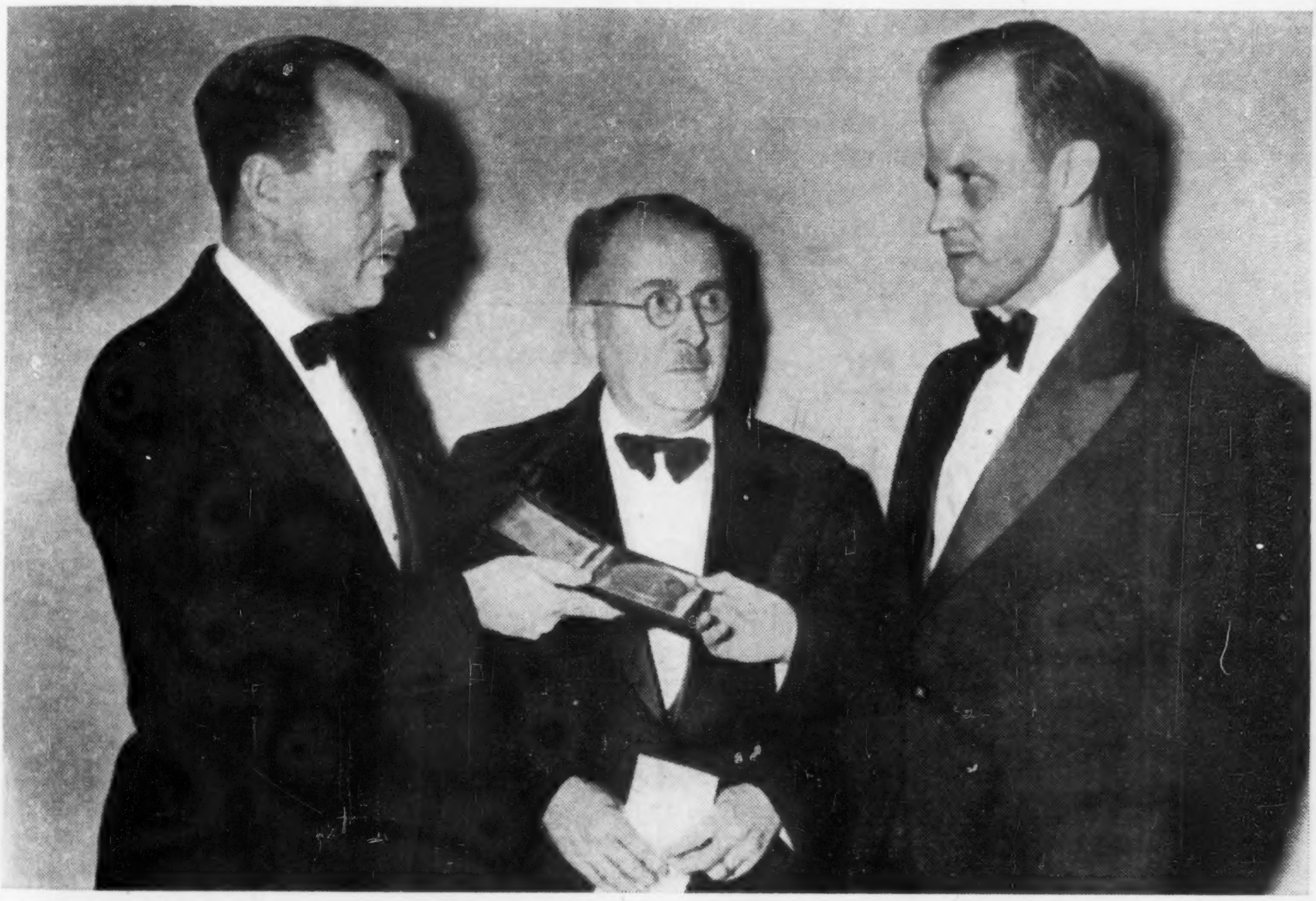
Vol. 105
No. 2739

Pages 653-666

June 27, 1947

Science

THE SCIENTISTS NEWSWEEKLY



Presentation of the 1947 Eli Lilly and Company Award in Bacteriology and Immunology, consisting of a medal and $1,000, to Wayne W. Umbreit, of Cornell University, at the 47th general meeting of the Society of American Bacteriologists in Philadelphia. The presentation was made by Thomas Francis, Jr., of the University of Michigan, president of the Society (*left*), and Leland W. Parr, of George Washington University, secretary-treasurer (*center*). The award is administered jointly by the American Society for Experimental Pathology, the American Association of Immunologists, and the Society of American Bacteriologists.

Published by the
AMERICAN
ASSOCIATION
FOR THE
ADVANCEMENT
OF SCIENCE

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Vol. 105 No. 2739 Friday, June 27, 1947

Science

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(*Cover photo by courtesy of the Philadelphia Inquirer.*)

Science a weekly journal, is published each Friday by the American Association for the Advancement of Science at Mt. Royal & Guilford Avenues, Baltimore 2, Maryland. Founded in 1880, it has been since 1900 the official publication of the AAAS. Editorial, Advertising, and Circulation Offices, 1515 Massachusetts Avenue, N.W., Washington 5, D. C. Telephone, EXecutive 6060 or 6061. Cable address SCIMAG, Washington, D. C.

Articles offered for publication should be sent to the Editor, 1515 Massachusetts Avenue, N.W., Washington 5, D. C.

Membership correspondence for the AAAS should be sent to the Administrative Secretary, 1515 Massachusetts Avenue, N.W., Washington 5, D. C.

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Annual subscription, \$7.50; single copies, \$.25; foreign postage (outside the Pan-American Union), \$1.00 extra, Canadian postage, \$.50 extra. Remittances and orders for subscriptions and for single copies, notices of changes of address, and claims for missing numbers should be sent to the Circulation Department.

Entered as second-class matter January 17, 1947, at the postoffice at Baltimore, Maryland under the act of March 3, 1879.

The Chemical Study of Penicillin:
A Brief History

The Editorial Board of the Monograph on the Chemistry of Penicillin

DURING THE DECADE FOLLOWING the discovery of penicillin (*1*) relatively little information was secured as to its chemical nature. Fleming had reported it to be soluble in water and in alcohol, but insoluble in ether or chloroform, and its thermal stability in solution to be maximal at neutrality. Three years later Raistrick and his collaborators (*2*) found that penicillin could be produced in a synthetic medium. They also showed that when solutions of penicillin at pH 7.2 were extracted with ether, some of the antibacterial activity was transferred to the ether, but that when the process was carried out at pH 2, the extraction was almost complete. They likewise noted that the antibiotic extract was sensitive to oxidants and was readily inactivated by evaporation in acid and alkaline solution, but moderately stable at pH 5–6.

After a further interval of three years Raistrick's findings were confirmed, in general, by Reid (*3*), who found, in addition, that the activity was lost on dialysis and that penicillin was adsorbed on charcoal.

In 1940 Chain, Florey, and their collaborators (*4*) prepared penicillin in solid, though inhomogeneous, form and reported on its effectiveness *in vivo* against various pathogenic organisms. In the same year Abraham and Chain (*5*) described the preparation from *B. coli* of an enzyme, penicillinase, which inactivates penicillin. In 1941 the Oxford workers (*6*) published details of a procedure for the concentration of penicillin from culture fluid obtained with the use of Raistrick's synthetic medium. This involved extraction from acid solution with organic liquids and further purification by chromatographic procedures. In this way there was secured (*7*) the barium salt of an acid which proved to be stable when absolutely dry or in organic solvents.

In 1942 Abraham and Chain (*8*), by further refinements of the process, succeeded in producing a barium salt with an activity of 450–500 units/mg. This product was later found (*9*) to contain nitrogen, but the provisional formula suggested, $C_{24}H_{32}O_{10}N_2Ba$, was soon found to be incorrect. One impurity detected was barium furoate. The significance of the nitrogen content was indicated by its linear relation to antibiotic potency. On hydrolysis this substance yielded carbon dioxide, a volatile acid, and the crystalline salt of a base. The authors recognized that their material was not homogeneous, so that these data could not be interpreted with certainty. It was also observed (*10*) that penicillin is inactivated by primary alcohols, by organic bases, and by various metallic salts—for example, those of copper and zinc.

The basic compound, referred to above, received the name penicillamine; it was shown (*11*) to be a primary amine and to contain one strongly and one weakly acidic group. The hydrochloride was at first assigned the formula $C_6H_{11}O_4N \cdot HCl$ and, later, $C_5H_{11}O_2SN \cdot HCl$. In so far as the Oxford work was concerned, the recognition of sulfur in penicillin (see below) followed directly from the study of an oxidation product of penicillamine. The analytical results indicated the uptake of three oxygen atoms and thus suggested conversion of a thiol to a sulfonic acid. The significance of penicillamine as an integral part of penicillin became obvious when it was obtained from penicillin having an activity of 1,000 units/mg. on the Oxford scale of that date.

In the meantime the study of penicillin was taken up in other laboratories. A product showing 750 units/mg. was obtained at the Imperial College of Science, London (*12*), where it was observed that penicillin, on degradation, yielded a product which appeared to be an amino acid. The conversion of penicillin in acid solution into a crystalline product termed penillic acid was recorded early in 1943 by chemists in the Wellcome Research Laboratories (*13*). In the United States, penicillin was obtained in the form of a crude ammonium salt (*14*), which had an activity of 240 units/mg. (*15*), but no evidence was presented as to its chemical nature.

On treatment with diazoalkanes, penicillin concentrates were found to yield esters which proved to be notably more stable than the salts. They showed little activity *in vitro*, but were antibiotically active *in vivo*. The benzhydryl ester was split by catalytic hydrogenation with regeneration of *in vitro* activity (*16*). It was later shown that the methyl and ethyl esters could be hydrolyzed to active penicillins by treatment with sodium hydroxide or sodium bicarbonate solution (*17*, *18*).

By 1943 recognition of the potential military importance of penicillin had led to restriction of chemical information on the subject. Investigation was continued with increasing intensity, in both academic and industrial research laboratories, but, in general, the results were privately communicated to other recognized workers in the field rather than to the scientific press. This exchange

From Chapter I of the monograph entitled *The chemistry of penicillin,* now in preparation under the supervision of the National Academy of Sciences and the Office of Scientific Research and Development, to be published by the Princeton University Press.

of information on the chemistry of penicillin was effected in Britain at first through unofficial conferences of interested workers and later by those sponsored by the Ministry of Supply and the Medical Research Council. There were also special agreements between certain pharmaceutical manufacturers in England (especially May and Baker, Ltd., one of the firms which entered the Therapeutic Research Corporation of Great Britain, Ltd.) and in the United States (Merck & Co., Inc., E. R. Squibb & Sons, and subsequently Chas. Pfizer & Co., Inc.). Information secured by the American firms was subsequently communicated to the Committee on Medical Research and disseminated through it; a similar procedure was followed in England, where the five firms participating in the Therapeutic Research Corporation, as well as Imperial Chemical Industries, Ltd., and various academic groups, reported to the Medical Research Council.

During the first half of 1943, progress in the chemical studies was made principally in Britain, where, in addition to penicillamine, 2-pentenylpenillic acid and 2-pentenylpenillamine were isolated as conversion products of the impure preparations then available. Experimental work in the United States was at first primarily directed toward problems of production and purification. It was found in the Northern Regional Research Laboratory and in the Merck and Squibb laboratories that chromatographic procedures, the efficacy of which had been demonstrated in Britain for the concentration of penicillins in the form of their free acids, could be applied advantageously to the more stable sodium salts. The partition chromatography of Martin and Synge was adapted for the purification and separation of the penicillins by chemists of Imperial Chemical Industries, Ltd. In the summer of 1943, MacPhillamy, Wintersteiner, and Alicino, of the Squibb group, succeeded (*19*) in crystallizing the sodium salt of benzylpenicillin. This important achievement, which made possible the accurate chemical study of the pure compound, immediately led to the recognition by the same investigators of sulfur as a constituent of the molecule. Coincidentally, the presence of sulfur in (impure) barium penicillin, as well as in penicillamine, penicillaminic acid, penillic acid, and other well-defined derivatives of penicillin, was discovered independently by the chemists in Oxford (*20*). Soon afterward the Oxford workers reported the crystallization of alkali metal salts of their penicillin.

At about the same time it became clear that the penicillin which had been obtained in crystalline form in the United States was not identical with the penicillin with which the British investigators had been working. Among other differences between the two was the far greater reluctance of the latter to crystallize. The chemical distinction between them was clearly brought to light during the middle of 1943 by observations in several quarters. One was the demonstration by Stodola, Wachtel, and Coghill, of the Northern Regional Research Laboratory, that the two varieties of penicillin give different, though analogous, crystalline derivatives when the free acids of the respective penicillins are treated with benzylamine. The second observation was the demonstration in the Merck laboratories, by analytical data on crystalline penillic acids, that there were at least two penicillins.[1] The American preparations had been found (*21*) to yield phenylacetic acid on hydrolysis, whereas the antibiotic studied in Britain yielded 2-hexenoic acid under similar conditions (*22*). Very convincing evidence was also obtained by the chemists of Imperial Chemical Industries, Ltd., at an early stage of the development. The main constituents of their own penicillin and of Merck penicillin were chromatographically separated (*23*). This was later confirmed by direct comparison of the derived penillic acids (*24*). It appears that these reports had a limited circulation.

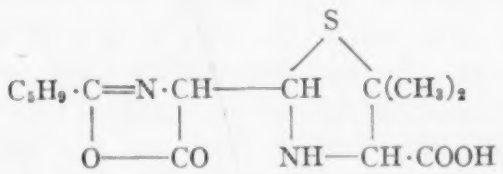
On the other hand, penicillamine having the same configuration was obtained from both types. After some uncertainty as to its constitution (*20*), penicillamine was recognized by the Oxford workers to be β,β-dimethylcysteine. This was demonstrated by chemical (*25*) and crystallographic comparison with a synthetic sample (*26*). Penicillamine had in the meantime been shown to yield thiazolidines on condensation with carbonyl compounds, and it was suspected that the same ring system was present in the penicillin molecule (*27*). Grounds for this suspicion had been furnished by the observation (*28*) that when the Oxford penicillin was decomposed by mercuric chloride, it yielded a carbonyl compound, probably an aldehyde, as well as penicillamine. This aldehyde was characterized in the form of a crystalline 2,4-dinitrophenylhydrazone and a condensation product with dimedone, and was shown to have the composition $C_8H_{13}O_2N$ (*27*). It was recognized as hexenoylaminoacetaldehyde and as the source of the glyoxal-osazones which were obtained early from mother liquors resulting after hydrolysis and separation of penicillamine. Meanwhile the Imperial College of Science group (*29*) had shown that the antibiotic studied in Britain could be reduced to a dihydro derivative which was biologically active and was recognized as a "natural" penicillin. It afforded n-caproic acid on hydrolysis. The position of the double bond in the hexenoic acid obtained from the unreduced antibiotic was determined at Oxford by permanganate oxidation to propionaldehyde. The Imperial College workers found that the penilloaldehyde from their dihydropenicillin afforded a dinitrophenylhydrazone which could be further changed to the glyoxal osazone. They therefore suggested that the aldehyde was n-caproylaminoacetaldehyde. In both series the identity of the penilloaldehydes was confirmed by synthesis.

[1] Systems of nomenclature using letters (in the United States) and Roman numerals (in Britain) were initiated late in 1943. However, in the interests of clarity and uniformity, workers in the field agreed early in 1946 on the nomenclature now in use, which involves a designatory prefix such as "benzyl."

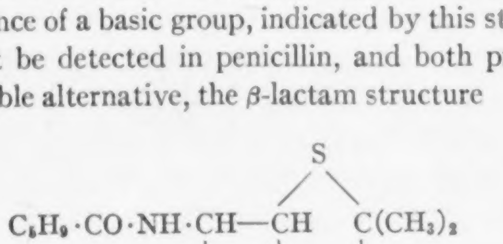
Analogous conclusions and hypotheses were attained independently and simultaneously by American workers. The chemists in the Squibb Institute (*30*) and in the Merck laboratories (*31*) produced evidence that the carbonyl compound secured by treatment of the American crystalline penicillin with mercuric chloride was phenylacetylaminoacetaldehyde. It was, for example, found to be oxidized to phenaceturic acid. At the same time the Merck group demonstrated that when the product formed by the action of benzylamine upon benzylpenicillin was treated with mercuric chloride and penicillamine was liberated, the benzylamine group remained in amide linkage in the residual portion of the molecule. A strictly analogous result was obtained with the methyl ester which resulted from the action of methanol upon benzylpenicillin. It was also shown by members of the Imperial Chemical Industries group (*32*) that the methyl ester of pentenylpenicillin, on treatment with mercuric chloride, yielded the methyl ester of penicillamine. The identity of the acid group in penicillin with the carboxyl group of penicillamine was thus definitely established by two independent methods.

In the recognition of degradation products of penicillin, invaluable aid was rendered by X-ray crystallographic measurements. For instance, the 2,4-dinitrophenylhydrazone of the penilloaldehyde secured in Oxford was shown by this method (*33*) to be identical with the hydrazone prepared from synthetic 2-hexenoylaminoacetaldehyde (*34*).

On the basis of these and many other findings, the Oxford workers proposed (*35*) the thiazolidine-oxazolone formula



as the simplest expression for their penicillin. An exactly corresponding formula was independently proposed for benzylpenicillin by the Merck, Squibb, and Abbott groups. However, the chemists in both the Oxford and the Merck laboratories drew attention to the fact that the presence of a basic group, indicated by this structure, could not be detected in penicillin, and both proposed, as a possible alternative, the β-lactam structure



At this point it was hoped that, as the penicillins were relatively simple compounds, synthetic methods for their production could be developed without much difficulty. The urgency of the need for large quantities of penicillin by the military forces made imperative the intensive exploration of this field on a wider front and on a basis of international collaboration. At the instance of the director of the Office of Scientific Research and Development and the chairman of the Committee on Medical Research in Washington, D. C., and the secretary of the Medical Research Council in London, the necessary diplomatic agreements were reached. The American group of collaborators was enlarged by the inclusion of 8 more industrial research laboratories and 10 academic laboratories.[2] Contracts between these organizations and the governmental agencies were entered into, according to the terms of which each contractor undertook to conduct experimental investigations in connection with the chemical structure of penicillin and the synthesis of penicillin or a therapeutic equivalent. All contractors undertook to report to their Government all pertinent information available to them at that time and thereafter to furnish monthly progress reports.[3] All of this information was transmitted as expeditiously as possible to each contractor in Britain and in the United States. In the United States the contracts, which began during December and January 1943–44, remained in force until November 1, 1945 in the case of the industrial organizations[4] and December 31, 1945 in the case of the academic institutions. In Britain, as already stated, the early collaboration was an informal by-product of a Penicillin Production Committee, sponsored by the Ministry of Supply. On January 1, 1944, the Medical Research Council set up a Committee on Penicillin Synthesis (CPS) under the

[2] The following groups collaborated in the general program: In America, the industrial participants were Abbott Laboratories; Cutter Laboratories; Heyden Chemical Corporation; Eli Lilly and Company; Merck & Co., Inc.; Parke, Davis and Company; Chas. Pfizer & Co., Inc.; Shell Development Company; Squibb Institute for Medical Research; The Upjohn Company; Winthrop Chemical Company, Inc. The academic and governmental participants were U. S. Department of Agriculture, Northern Regional Research Laboratory; Cornell University Medical College, Department of Biochemistry, and Russell Sage Institute; Federal Security Agency, Food and Drug Administration; Harvard University, Department of Chemistry; University of Illinois, Department of Chemistry; University of Michigan, Departments of Chemistry and Physics; National Bureau of Standards; Naval Medical Research Institute; The Rockefeller Institute for Medical Research.

In Britain the industrial participants were Boots Pure Drug Company, Ltd.; British Drug Houses, Ltd.; Glaxo Laboratories, Ltd.; Imperial Chemical Industries, Ltd. (Alkali Division); Imperial Chemical Pharmaceuticals, Ltd.; May and Baker, Ltd.; Wellcome Foundation, Ltd. The academic and governmental participants were Cambridge University, Departments of Chemistry and Colloid Science; Imperial College of Science, London, Department of Organic Chemistry; The London Hospital Medical Unit; Manchester University, Department of Chemistry; National Institute for Medical Research, Hampstead, London; Oxford University, Department of Crystallography, Dyson Perrins Laboratory, Sir William Dunn School of Pathology, and the Department of Physical Chemistry.

[3] Arrangements have been made for the deposition of a complete file of these reports with the U. S. Department of Commerce, Office of Technical Services, from which reproductions of desired portions can be obtained on request. Copies of these reports are also being filed in scientific libraries in Britain.

[4] The industrial participants in the chemical projects performed the subject work of their contracts without financial aid from their Governments. In both countries, however, grants were made to the academic groups in support of their work.

chairmanship of Sir R. Robinson and including representatives of the industrial groups, academic centers, and the Ministry of Supply. The regular exchange of British and American reports then began, but because of delay in the first months much work was duplicated. Attention is drawn elsewhere to the more important consequences of this situation.

The unrestricted exchange of current information at frequent intervals resulted in as close a collaboration as is possible among a widely distributed group of laboratory teams, but no attempt was made to avoid duplication of effort in the various laboratories. In consequence it is difficult or impossible, except in a relatively few specialized phases of the joint effort, to assign sole scientific credit for individual findings secured during the period covered by the contracts.[5] In the outline that follows no attempt is made to do more than touch upon some of the more significant results.

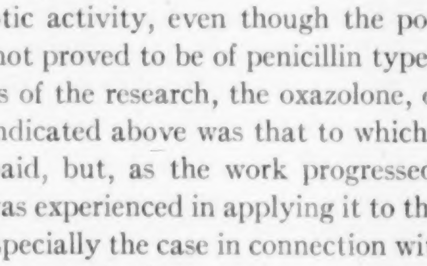
In the winter of 1943–44, an important problem was the confirmation and clarification of the structure assigned to penicilloic acids, the primary product of the hydrolysis of penicillins. The major part of the light thrown on this problem was supplied by the Merck laboratories. The D (or "unnatural") configuration of penicillamine was established by successive treatment with phenylisocyanate and with Raney nickel catalyst, whereby the sulfur atom was replaced by hydrogen; the product was identical with the phenylureide derivative of D-valine (*31*).

As stated in *Science* (*36*), the constitution of the penaldic acid, with which penicillamine is combined in benzylpenicilloic acid, was established (*31*) by its conversion on catalytic hydrogenation into cyclohexylacetylalanine. Under less drastic conditions of hydrogenation corresponding derivatives of serine were obtained. As these amino acid derivatives were optically inactive, they yielded no information as to the configuration of that part of the penicilloic acid molecule. This information was secured only later, when it was shown in the Merck laboratories (*37*) that under suitable conditions of hydrogenation derivatives of L-alanine, which has the "natural" configuration possessed by the amino acids of proteins, were formed.

Derivatives of penaldic acid were synthesized in several laboratories by the condensation of ethyl formate or orthoformate with esters of phenylacetyl glycine, and when the products were condensed with synthetic D-penicillamine, esters of penicilloic acids were obtained. In a very complete study, in the Merck laboratories (*38*), methyl esters of three of the four theoretically possible diastereoisomers of the penicilloic acids derived from D-penicillamine were synthesized, and it was shown that at least two of these were formed by the action of methanol upon benzylpenicillin. Somewhat later, esters of the fourth isomeric form of penicilloic acid were synthesized in the Squibb Institute (*39*).

It has already been mentioned that the conversion of penicillins into penillic acids contributed largely to the recognition of the existence of more than one variety of penicillin. The constitution of benzylpenillic acid was confirmed by synthesis in two laboratories during 1944. An optically inactive monomethyl ester was prepared by Cook, Elvidge, and Heilbron (*40*) by condensing the methyl ester of phenylthioacetyldiethoxyalanine with DL-penicillamine. The dimethyl ester of optically active penillic acid, identical with the product from benzylpenicillin, was synthesized by the Merck group (*41*) by condensing the Schiff base from N-formyl-α-formylglycine methyl ester and benzylamine with the methyl ester of D-penicillamine.

Attempts, made in many laboratories, to produce penicillins by anhydrization of penicilloates met with almost universal failure. It is worthy of note, however, that they did provide the first synthetic material possessing antibiotic activity, even though the potencies were small and not proved to be of penicillin type (*42*). In the early stages of the research, the oxazolone, or azlactone, structure indicated above was that to which most attention was paid, but, as the work progressed, increasing difficulty was experienced in applying it to the penicillins. This was especially the case in connection with the physicochemical investigations such as the electrometric studies by Neuberger (*43*) and many others. A number of structures which had been proposed at the outset were excluded, during the first half of 1944, by the observation that the penicillin molecule contains only one labile hydrogen atom other than that of the carboxyl group. This was indicated in the Abbott laboratories by the action of Grignard reagent upon methyl benzylpenicillinate (*44*) and at Cornell University by equilibration of sodium benzylpenicillinate with deuterium oxide (*45*). The structural limitations thereby imposed were met by the formulas set forth above, as well as by a tricyclic formula



[5] Shortly after the contracts between OSRD and the industrial participants had expired, a brief summary announcement of the principal findings was published in *Science* (*36*) and in *Nature* (1945, **156**, 766). At a conference, attended by scientific representatives of all the cooperating groups, held on January 9, 1946, it was decided that detailed reports of the results secured under the collaborative program should, in general, be published in a monograph rather than in individual papers in the scientific press. However, provision was made for the publication, in advance of the monograph, of papers which, in the opinion of the Editorial Board, would not conflict with the plan. Several such articles have appeared.

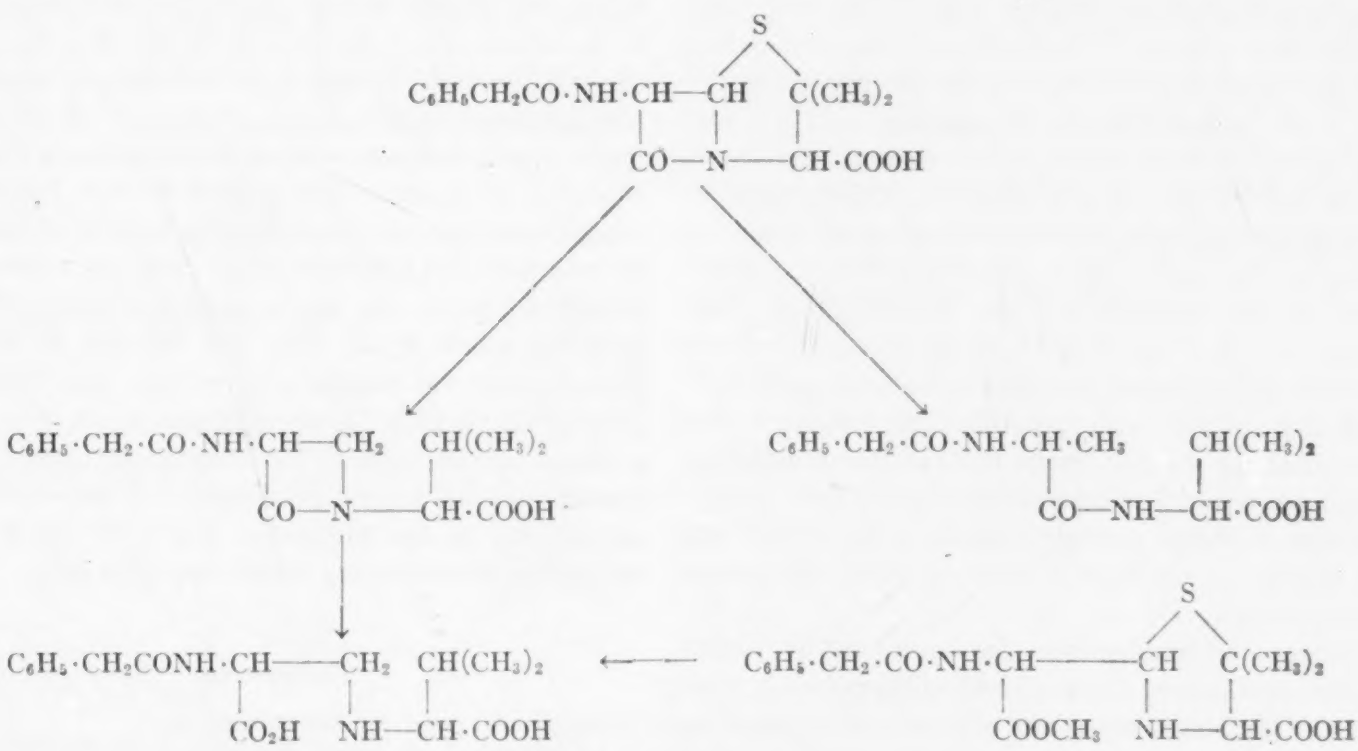
When the monograph was planned, it was hoped that its writing could be completed in six months; unfortunately, this estimate proved unduly optimistic.

which had been proposed by several of the participant groups.

Among these alternatives, the β-lactam structure gradually gained popularity. This formula received support from physicochemical evidence. Infrared absorption spectra were studied principally by the Shell Development Company; Thompson, of Oxford University; Sutherland, of Cambridge University; Randall and collaborators, of the University of Michigan; Merck & Co., Inc.; and the Russell Sage Institute, Cornell University Medical College. An important result was the demonstration by the Shell Development Company of the presence in penicillins of a distinctive absorption band which also appeared in three models containing a fused β-lactam-thiazolidine structure, prepared in its laboratories. Also, other absorption bands found in the spectrum of penicillin were considered to indicate the presence of a monosubstituted amide group. The models here show that the bands extend over a certain range and that the penicillin band is on the margin of this range. Thus, the amide group is perhaps present in a modified form.

all three salts in February and March 1945; it was later fully confirmed by the evaluation of the electron density in three dimensions for the sodium and potassium salts alone. The detailed arrangement of the atoms found in these salts determined not only the essential chemical formula but also the stereochemical relations between the different groups within the penicillin molecule. X-ray spectrum determinations, made by Clark and his group at the University of Illinois, contributed greatly to our knowledge of the various penicillins and their derivatives.

From the standpoint of organic chemistry, the most convincing evidence was secured by a study, carried out in the Merck laboratories (*46*), of the action of Raney nickel catalyst upon sodium benzylpenicillinate. Two products resulted; one was benzyldesthiopenicillin, a monocarboxylic acid $C_{16}H_{20}O_4N_2$, which on hydrolysis yielded D-α-benzyldesthiopenicilloic acid, identical with the product of the action of Raney nickel catalyst on α-methyl D-α-benzylpenicilloate; the other was a monocarboxylic acid $C_{15}H_{22}O_4N_2$, identified with phenylacetyl-L-alanyl-D-valine (see formula given directly below).



Thermochemical data, secured by the National Bureau of Standards and interpreted by Woodward, of Harvard University, suggested the presence of a strained system in penicillin, comparable with that of authentic β-lactams. More cogent still, the CNOS skeleton of the penicillin molecule was conclusively demonstrated through the X-ray crystallographic analysis of sodium, potassium, and rubidium benzylpenicillins by Crowfoot and Low (Oxford University) and by Bunn and Turner-Jones (Alkali Division, Imperial Chemical Industries, Ltd., Northwich). The existence of the β-lactam ring was first shown clearly in electron density projections derived from

As sodium benzylpenicillinate, when similarly treated in the absence of Raney nickel catalyst, underwent no loss of antibiotic activity or change in optical rotation, it may be assumed that these products were formed without intramolecular rearrangement.

In studies of the interaction of thiocyanate and benzylpenicillin, begun in October 1944 and carried on throughout 1945 in the laboratories of Cornell University Medical College and the Squibb Institute, it was found that whereas azlactones in general yield 2-thiohydantoins, penicillin does not do so, but resembles an authentic β-lactam, studied by the Merck group (*47*), in yielding a

thiohydrouracil. Although the oxazolone and β-lactam structures would give a common intermediate with thiocyanate, the results of this intricate investigation may be considered to support the lactam structure. The properties of the sulfur atom in penicillin were also found to accord with this hypothesis. Thus, methyl benzylpenicillinate affords a sulfone (*48*) and a sulfoxide (*49*). Hence, penicillin behaves like an acylated thiazolidine (*50*), in contrast to unacylated thiazolidines, in which the ring is opened on oxidation.

During the period in which the penicillin molecule was considered to contain the oxazolone (azlactone) ring, many attempts were made to synthesize penicillin-like compounds by condensing appropriate alkoxymethylene oxazolones with penicillamine. Experiments conducted in the Merck laboratories (*51*) showed that a trace of antibiotic activity corresponding to benzylpenicillin could be produced by this general method. It was independently found in Oxford (*52*) that a similar small degree of antibiotic activity was produced in a reaction designed to yield an artificial "styrylpenicillin." The work was extended to other cases including propyl-, *n*-amyl-, benzyl-, and phenylpenicillins, and similar antibiotic activity was obtained. The chemists of May and Baker, Ltd., also condensed 2-phenyl-4-ethoxymethylene oxazolone with DL-penicillamine in pyridine solution and obtained an antibiotic product (*53*). It was later shown by the Oxford workers (*54*) that this activity was destroyed by penicillinase, and it was therefore attributed to a compound of the penicillin type. Support for this interpretation was supplied by the Cornell group, who showed (*55*) that the mixture of the reaction products formed in the condensation of 2-benzyl-4-methoxymethylene-5(4)-oxazolone with penicillamine exhibited the same quantitative relationships in its antibiotic activities toward a series of 7 microorganisms as did pure benzylpenicillin. Evidence strongly suggestive of identity was also secured by the Cornell workers, using the isotope dilution method (*56*).

Attempts to concentrate the antibiotically active product synthesized by the method described a year earlier by the Merck group met with only partial success; chromatographic procedures used by the Upjohn chemists (*57*) and countercurrent distribution technique used at Cornell (*58*) led at best to preparations containing less than 3 per cent and 17 per cent of penicillin, respectively.[6] Although this method of synthesis was based on the thiazolidine-oxazolone structural theory, it must be pointed out that a rational synthesis of the β-lactam structure by way of the oxazolone is conceivable as the result of intramolecular acylation.

[6] At this point the contracts expired; the investigation, continued by the Cornell group as an independent project, led to the isolation, early in 1946, of synthetic benzylpenicillin in crystalline form. An account of the background and results of this work, together with full references, was, at the request of the Editorial Board of the monograph on the chemistry of penicillin, published in November, 1946 (*Science*, 1946, **104**, 431).

Concurrently with the studies of structure, the search for other penicillins was carried on in several laboratories. The principal accomplishments in this line of endeavor were proof of the existence of p-hydroxybenzylpenicillin by the isolation of the corresponding penillic acid and related compounds in the Imperial College of Science (*59*), followed by the isolation of the antibiotic itself in the Northern Regional Research Laboratory (*60*), of n-heptylpenicillin in the Abbott Laboratories (*61*), of flavacidin (probably 3-pentenylpenicillin) from *Aspergillus flavus* in the Squibb Institute for Medical Research (*62*), and the production at the Northern Regional Research Laboratory of halogenated penicillins and aryl azopenicillins by the action of halogens and of diazo compounds upon p-hydroxypenicillin (*63*).

A development of great technical importance resulted from the observation, first recorded by the Northern Regional Research Laboratory (*64*), that the addition of phenylacetic acid and related compounds to media in which penicillin is elaborated in surface culture gives rise to increased yields of antibiotic products. Attempts to induce a similar effect with submerged cultures having failed, the chemists of the Lilly Research Laboratories, in an extensive survey, discovered (*65*) that the yields of penicillin could be raised by the addition of phenylacetyl derivatives of various L-amino acids. It was shown that a strain of *P. notatum* which had hitherto not been observed to produce any antibiotic but 2-pentenylpenicillin actually produced benzylpenicillin when grown in presence of phenylacetamide (*66*); in presence of p-hydroxyphenylacetic acid a yield of p-hydroxybenzylpenicillin much higher than any isolated in previous fermentations was obtained. This was a clear indication of biosynthesis (*67*). Extension of this principle to analogous compounds, in which the benzyl group of the phenylacetamino acids was replaced by a wide variety of other groups, led to the production and isolation in pure, crystalline form of many novel penicillins (*68*).

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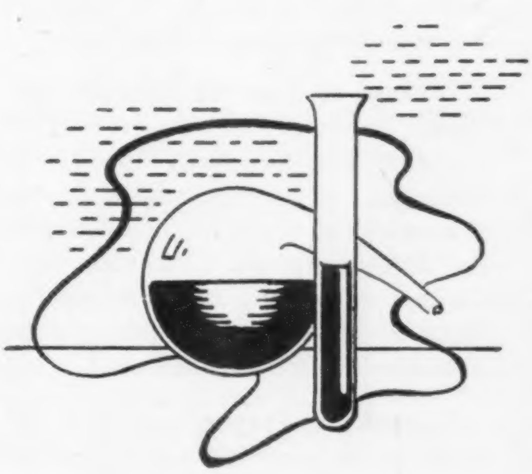

NEWS
and Notes

A part of the annual exhibition of the Photographic Society of America has, for the past two years, been devoted to a section showing scientific and technical photographs. At the 1946 meeting of the Association more than 200 such prints were hung in the section, and about 50 of these were selected and made available to technical societies, camera clubs, etc. This year the annual meeting and exhibition will be held in Oklahoma City. Readers of *Science* who wish to submit prints for the technical section should secure complete information from W. F. Swann, 343 State Street, Rochester 4, New York. The final date for receipt of prints is September 8. Subject matter for the section may cover any phase of technical photography except pictorial photographs of technical and mechanical operations. Both black-and-white and color photographs are acceptable, and there is no limit to the number of prints which may be submitted by any one individual.

Section Q (Education) especially desires that reports of research evaluating the educational product of the present generation as compared with earlier ones be presented at the Chicago meetings. A limited number of papers on other topics will be considered. Those wishing to submit papers should send them to D. A. Worcester, Secretary of the Section, University of Nebraska, Lincoln 8, Nebraska.

About People

H. J. Muller, professor of genetics, University of Indiana, and Nobel Prize winner in physiology and medicine, has been elected a trustee of the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts.

Russell A. Huggins, assistant professor of pharmacology, University of Georgia Medical School, has been appointed associate professor of pharmacology, Baylor University College of Medicine, and will assume his duties there on July 1.

Wendell F. Hess, head of the welding laboratory, Rensselaer Polytechnic Institute, has been appointed head, Department of Metallurgical Engineering, to succeed Matthew A. Hunter, who will continue as dean of the faculty.

Dennistoun W. Ver Planck, professor of electrical engineering, Carnegie Institute of Technology, has been appointed professor of mechanical engineering and head, Department of Mechanical Engineering, effective September 1. Dr. Ver Planck will assume the position last held by Willibald C. L. Trinks, who retired in 1943.

Stanhope Bayne-Jones has resigned as professor of bacteriology, Yale University, and director, Board of Scientific Advisers, The Jane Coffin Childs Memorial Fund for Medical Research, to become the first president of the Joint Administrative Board, New York Hospital-Cornell Medical Center. After July 1 Dr. Bayne-Jones will maintain offices at 525 East 68th Street, New York City.

Alfred Gilman, College of Physicians and Surgeons, Columbia University, gave the annual Alpha Omega Alpha Lecture at Western Reserve University School of Medicine, on the topic, "The Contributions of Chemical Warfare Research to Medicine."

Edward U. Condon, director, National Bureau of Standards, and **Detlev W. Bronk,** chairman, National Research Council, have been appointed members of the scientific advisory committee of the Brookhaven National Laboratory.

Howard S. Gardner, associate professor, and chairman, Department of Chemical Engineering, University of Rochester, has been appointed director of research and development, Fibreboard Products, Inc., San Francisco. Dr. Gardner will head the firm's new Research and Development Division to be established in Antioch, California.

Russell E. Teague has been assigned to the Henry Phipps Institute of the University of Pennsylvania by the Tuberculosis Control Division of the U. S. Public Health Service, where he will serve as assistant director of the Institute and assistant professor of public health in the University. Dr. Teague will continue as consultant on tuberculosis for District No. 1, U. S. Public Health Service.

Visitors to U. S.

E. C. Marais, of the National Physical Laboratory (South African Council for Scientific and Industrial Research), arrived in Washington, D. C., June 16. Dr. Marais is to work at the National Bureau of Standards.

Alan Robertson, of the Agricultural Research Council's National Animal Breeding Research Organization, and Mrs. Robertson are visiting the United States. Dr. Robertson, traveling under a research fellowship, will study the latest techniques in animal genetics.

T. Goodey, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine, has arrived in the United States to spend three months observing U. S. research in nematology. Dr. Goodey is visiting this country on a traveling research fellowship awarded by the Ministry of Agriculture.

E. T. Jones, of the Welsh Plant Breeding Station, will visit the United States and Canada under a Ministry of Agriculture traveling fellowship to study application of newer techniques of plant breeding.

Colleges and Universities

Massachusetts State College, Amherst, in May became the University of Massachusetts. This change in name is the third since the institution was founded in 1863. On the 700-acre campus in Amherst are five schools and three divisions in the undergraduate college, a graduate school which has been organized as a separate unit since 1908, and a two-year school of agriculture. A $3,000,000 building program, now in progress, includes new dormitory projects, a new physics building, a school of home economics, an animal pathology laboratory, and classroom buildings.

Charles University, Prague, Czechoslovakia, has opened a new Department of Parasitology and Protozoology which is headed by Otto Jírovec. The Department, located at Praha II, Viničná Nr. 7, has published 10 scientific works in Czech, Swiss, and English journals since the end of the war. At present, research

work is being done on the chemotherapy and epidemiology of *Trichomonas vaginalis* and on the epidemiology of Leptospirosis. In addition, the Department may be called upon for all diagnostic work on parasitic diseases of the country. Dr. Jírovec reports that the Department still lacks modern parasitological literature and would appreciate receiving such literature, especially in the form of reprints.

Meetings

Centenary Celebrations of the Chemical Society, London, the oldest chemical society in the world, will be attended on July 15–17 by representatives of about 20 countries. The American Chemical Society, the world's largest, is sending as its official delegates its president, W. A. Noyes, Jr.; its president-elect, C. A. Thomas; and its secretary, A. H. Emery. Also representing the United States will be Marston T. Bogert, president of the International Union of Chemistry, and Linus Pauling, one of the Society's Honorary Fellows.

The Tri-State Field Conference, participants in which are the staff members and graduate students of the various universities, colleges, and State Geological Surveys in Wisconsin, Iowa, and Illinois, will be held early in October. W. H. Twenhofel, professor of geology, University of Wisconsin, will lead the group on a two-day trip through the Silurian of eastern Wisconsin. Further information may be obtained through L. M. Cline, Department of Geology, University of Wisconsin, Madison 6, Wisconsin.

The annual Field Conference of Pennsylvania Geologists, held at Lehigh University May 30–June 1, was attended by about 80 geologists from Pennsylvania and neighboring states. An afternoon field trip was conducted on May 30 by Lawrence Whitcomb and H. R. Gault to the abandoned zinc mines of the Saucon Valley and to an area of Triassic basic intrusives south of Bethlehem. On May 31 the Conference participated in an all-day field trip up the valley of the Lehigh River. This trip, led by Bradford Willard and Lawrence Whitcomb, covered the entire Paleozoic sequence from the pre-Cambrian into the coal measures. In addition to the stratigraphic sequence, the physiographic and structural features were discussed and a visit was made to Wild Creek Reservoir, which supplies the water for the City of Bethlehem.

Two alternate trips were offered on the morning of June 1. Prof. Willard led a party to the Delaware Valley to observe the Triassic fanglomerates and their relation to the Paleozoics there, while Prof. Whitcomb conducted a trip to the Spitzenberg, about 25 miles west of Bethlehem, for the purpose of observing its peculiar stratigraphy and discussing its bearing upon certain physiographic problems.

A dinner was held at the University on the evening of the 31st. At a business meeting the previous evening, it was unanimously voted to accept the invitation of S. H. Cathcart, director of the Pennsylvania Bureau of Topographic and Geologic Survey, to hold the 1948 meeting at Harrisburg.

Bradford Willard, head, Department of Geology, Lehigh University, was chairman of the 1947 meeting.

An International Short Wave Congress, one of the first international medical congresses since the end of the war, will be held in Amsterdam, July 19–24, 1948. Biologists and physicists in the field of short-wave therapy who are interested in submitting papers are invited to communicate with H. Th. Boersma, Secretary of the Foreign Correspondence Department, Meyendelscheweg 2, Wassenaar, The Netherlands. Manuscripts must be in the hands of the principal secretary, Dr. J. Samuels, Weteringschans 73, Amsterdam, before April 15, 1948.

Members of the board responsible for direction of the Congress are: W. Beaumont, London; A. Gjertz, Stockholm; C. Guarini, Naples; D. Kobak, Chicago; W. Kowarschik, Vienna; P. Liebesny, New York; J. Meyer, Paris; L. Rósa, Budapest; J. Saidman, Paris; J. Samuels, Amsterdam; F. Scheminzky, Innsbruck; and E. Schliephake, Würzburg.

Recent Deaths

George Eric Macdonnell Jauncey, 58, professor of physics, Washington University, St. Louis, Missouri, died May 19 at his home in St. Louis. Dr. Jauncey, who had been a member of the physics staff since 1920, was well known as an authority in the field of X-ray scattering.

Alice Cary Atwood, 70, formerly botanical bibliographer, U. S. Department of Agriculture Library, co-author of *Geographical guide to floras of the world* and largely responsible for the development of the Botanical Subject Catalog in the Library, died in Washington on May 20.

Warren H. Meeker, 79, professor and head of the Department of Mechanical Engineering, Iowa State College, from 1907 to 1934, died May 30 in Mary Greeley Hospital in Ames.

Eben J. Carey, 57, dean, Medical School, Marquette University, died June 5 of a liver infection in Columbia Hospital, Milwaukee, Wisconsin.

James Henri Walton, 69, professor of chemistry, University of Wisconsin, since 1919, and a member of the Department of Chemistry since 1907, died June 6 after an extended illness.

Karol Bohdanowicz, 82, director of the National Geological Institute, Warsaw, Poland, died June 7.

Arthur D. Emmett, 68, formerly assistant director, Research Laboratories, Parke, Davis & Company, died of pneumonia June 11, in Jennings Hospital, Detroit, Michigan.

The Naval Engineering Experiment Station, Annapolis, Maryland, has added to its Engineering Council a statistician to advise the director and various laboratory superintendents in the utilization of modern statistical techniques, and to perform statistical analyses as necessary. This program, introduced by Adm. D. H. Clark and having the support of the new director, Capt. W. D. Leggett, Jr., is the first of its kind installed in Bureau of Ships laboratories. Miss Besse B. Day, formerly of the Applied Physics Laboratory, Johns Hopkins University, has been appointed to the position.

The staff of the Philippine Fishery Rehabilitation Program of the U. S. Fish and Wildlife Service left San Francisco, California, early this month aboard the *Spencer F. Baird* and the *Theodore N. Gill*, recently commissioned research vessels. This program, one aspect of the provisions of the Tydings Act (Public Law 370 of the 79th Congress), is designed to continue to June 30, 1950 and will be concerned with biological, oceanographic, and technological problems connected with the revival of the large Philippine fish industry.

Offices and the main laboratories are established in Manila, but a forwarding agent will be maintained in San Francisco. The address of the project will be: ℅ Philippine Fishery Program, U. S. Fish and Wildlife Service, 100 Old Mint Building, Fifth and Mission Streets, San Francisco 3, California.

The scientific program is divided into two main subdivisions, one involving biological and oceanographic studies and another for technological research.

The scientific staff of the biological and oceanographic program includes: Herbert E. Warfel, aquatic biologist, formerly with the Bingham Oceanographic Laboratory, Yale University, in charge of biological and oceanographic investigations; Albert W. C. T. Herre, former curator of Ichthyology, Stanford University, consulting ichthyologist assigned to complete a check-list of Philippine fishes; Joseph Goodman, lately with the Department of Aviation Medicine, University of California School of Medicine, and formerly with the California Academy of Science, oceanographic chemist and chief-of-party on the *Spencer F. Baird*; William E. Wood, recently with the Scripps Institution of Oceanography and formerly with the Woods Hole Oceanographic Institution, physical oceanographer on the *Spencer F. Baird*; Earl S. Herald, lately ichthyologist of Operation Crossroads, U. S. Army, Washington, D. C., and one-time biologist with the California Fish and Game Department, biologist on the *Spencer F. Baird*; Charles B. Wade, until recently district biologist with the Central Valley Fisheries Studies of the U. S. Fish and Wildlife Service, Antioch, California, and one-time curator of Fishes at the Allan Hancock Foundation, University of Southern California, biologist on the *Spencer F. Baird*; Donald E. Kauffman, formerly fisheries biologist with the Salmon Division, Libby, MacNeill and Libby, Seattle, Washington, biologist and chief-of-party on the *Theodore N. Gill*; Ralph E. Jentoff, recently teaching fellow in the Department of Chemistry, University of Washington, Seattle, chemist on the *Theodore N. Gill*; William F. Carbine, formerly biologist with the Michigan Institute of Fisheries Research, Ann Arbor, biologist and chief-of-party in pond-fish research; Gilbert E. Wardwell, formerly with the Waterfowl Depredation Program of the U. S. Fish and Wildlife Service, Sacramento, California, biologist in pond-fish research; and Edward E. Cowles, lately an instructor in chemistry at Aberdeen Junior College, Aberdeen, Washington, chemist in charge of the shore laboratory.

The technological personnel of the program comprises: John A. Clague, formerly manager of the Food Engineering Division, Maxson Food Systems, Queens Village, New York, and biochemist with the National Canners Association, in charge of technological and bacteriological studies; William Hamm, formerly in charge of the Puerto Rico Fisheries Technological Laboratory of the Fish and Wildlife Service and lately with the Boston Laboratory of the same service, in charge of studies on fresh and processed fish; Robert Berueffy, formerly with the Ketchikan Laboratory of the Fish and Wildlife Service, Ketchikan, Alaska, in charge of vitamin and reduction investigations; William Arcisz, formerly in the Fish and Wildlife Service Laboratory, College Park, Maryland, bacteriologist; Arthur C. Avery, recently carrying on food research at the University of Massachusetts, Amherst, assistant in processing studies; Robert K. Pedersen, recently technologist with the State Department of Fisheries, Seattle, Washington, assistant in processing studies; Charles Rogers, formerly with the Feed and Fertilizer Laboratory, University of Massachusetts, assistant in vitamin studies; and Harry Hinkle, formerly with the Market News and Development Office of the Fish and Wildlife Service at San Pedro, California, fisheries economics.

The U. S. Atomic Energy Commission has announced that concentrated Boron 10 is available in a limited quantity for general distribution. Boron 10 will be packaged for shipment in the form of the solid complex boron trifluoride-calcium fluoride. The complex contains 6.9 per cent elemental boron, of which 96 per cent is B^{10}. Approximately 6.5 grams of $BF_3 \cdot CaF_2$ are needed to obtain one liter of BF_3 at normal temperature and pressure (assuming 100 per cent liberation). The boron trifluoride may easily be released as a gas by heating to temperatures above 260°C. in a vacuum. Organic vapors and air released from the complex during heating will be present in the BF_3 and will have to be removed in processing material for use in neutron counters. Information on a process which has proved satisfactory for the distillation of BF_3 from the complex will be available after July 1, 1947.

The price of $BF_3 \cdot CaF_2$ complex is $2 per gram independent of quantity. Shipping charges (express or postage) will be added to the invoice. There is no additional handling fee per shipment. Standard units of 1, 5, 10, and 50 grams have been packaged in glass containers with moisture-proof plastic screwtops.

An allocation of B^{10} may be applied for by submitting a completed "Stable Isotope Request," AEC Form 100, in quadruplicate to the U. S. Atomic Energy Commission, Oak Ridge, Tennessee, Attention: Isotopes Branch. Requests will be carefully reviewed and allocations will be restricted to reasonable quantities for the proposed investigation. Clinton Laboratories, Monsanto Chemical Company, P. O. Box 1991, Knoxville 11, Tennessee, will act as the supplier.

The National Bureau of Standards has developed a new method for isotope separation known as countercurrent electromigration, which makes use of the difference in the ionic mobilities of the isotopes of an element. It has been developed to a point where it can be used as a practical means of separating ionic species in general. The main advantage of this method over alternative methods is the simplicity of the apparatus. Isotopic separation takes place in a single step without the need of a vacuum system, and with automatic controls the system becomes entirely self-regulating. The process has the added convenience of being well adapted to use with many elements which may easily be obtained in ionic solutions.

Make Plans for—

Fifth International Pediatrics Congress, July 14–17, Waldorf-Astoria Hotel, New York City.

American Veterinary Medical Association, August 18–21, Cincinnati, Ohio.

American Pharmaceutical Association, August 24, Milwaukee, Wisconsin.

American Society of Mammalogists, August 24–27, Higgins Lake, Michigan.

American Institute of Electrical Engineers, Pacific General Meeting, August 26–29, San Diego, California.

TECHNICAL PAPERS

A Principle for Maintaining Earthworms in Farm Soils

Henry Hopp and Paul J. Linder[1]

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Ever since the classic work of Darwin (*2*), evidence has been accumulating that earthworms have important physical and chemical effects on soils (*1, 3, 5*). This information has not been of much direct use in row-crop farming because practical measures have not been devised for maintaining earthworms on clean-tilled land. In recent studies (*4*) on a Maryland soil, the earthworm population was found to be much lower under annual clean-tilled crops than under sod crops. The reason for this difference has since been investigated, with the purpose of finding a practical means of avoiding the decline in earthworms on annually clean-tilled land.

The investigation was conducted on previously established agronomy plots at the Maryland Agricultural Experiment Station (*4*). Monthly, from February 1946 to January 1947, the earthworms in the plow layer were counted. Table 1 gives the results for plots cropped to corn annually with and without a winter cover and to a two-year rotation in which corn is followed by various legumes and grasses. In plots cropped to corn annually the earthworms increased during the growing season until early November. Between November 23 and December 7 a sudden cold period occurred, and most of the earthworms were killed at this time.

TABLE 1

Earthworm Population by Seasons in Continuous Row-Cropping and in Two-Year Rotations

Season	Earthworms (thousands/acre)		
	Corn annually	Two-year rotation	
		Sod in spring, corn in summer, wheat in fall	Wheat in spring, sod in summer and fall
Early spring	60	290	85
Late spring	140	290	80
Summer	125	300	75
Early fall	240	440	305
Late fall (after freeze)	85	85	335
Winter	80	60	320

During this cold period many of the earthworms in the rotation plots containing young wheat also died. In the rotation plots that contained a sod of wheat stubble plus legumes and grasses, the earthworms survived.

[1] The collaboration of Homer T. Hopkins, formerly associated with this project, is acknowledged with pleasure. Aggregate analyses were made by Jay C. Bryant.

At a nearby weather station, a minimum air temperature of 8° F. was reached at this time. On land that was bare or contained young winter wheat, the soil froze to a depth of about 4 inches; but under the sod, and in other plots covered with straw mulch, no freezing occurred. When the plots were resampled between December 4 and 10, numerous dead earthworms were found in those with frozen soil.

TABLE 2

Change in Body Weight According to Soil Temperature

Soil temperature (°F.)	Change in body weight (%)
32	−100
36	+3
40	−6
50	−15
55	−6
60	−22
70	−19
80	−58

The response of earthworms to temperature was checked further in the laboratory. Earthworms from a sod field were placed in jars of soil having 40 per cent moisture content. The jars were kept at various temperatures in cold storage chambers. The earthworms did best at 36° F., as judged by changes in body weights, but died at 32° F. (Table 2). In a subsequent test in which earthworms from the various agronomy plots were subjected to a temperature of 32° F., all died, regardless of the plots from which they had been taken.

TABLE 3

Effect of Surface Protection on Winter Survival of Earthworms

(January 21, 1947)

1946 crop	Type of surface protection	Earthworms (thousands/acre)
Corn	None	0
	Burlap	995
Corn	None	0
	Lespedeza mulch	1,610
Soybeans	Light residue	335
	Heavy residue	665

These observations indicate that the death of earthworms on the clean-tilled land in the late fall was induced by the sudden drop in temperature to below 32° F. According to observations on three of the plots, this harmful effect can be alleviated by surface protection. These plots were almost bare except for small areas that had been protected with surface coverings of lespedeza hay, soybean residue, and burlap, respectively. The earthworms under the coverings withstood the cold and were highly active (Table 3).

Various practical ways of protecting the soil surface against sudden drops in temperature in the late fall may be suggested. Threshing residues, chopped corn stalks, composts, or manure might be spread early in the fall after the row crop has been removed. Where these materials are not available, a fast-growing catch crop that forms a winter mulch might be interseeded at the last cultivation of the row crop.

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Distribution of C^{14} in Photosynthesizing Barley Seedlings[1]

S. Aronoff, A. Benson, W. Z. Hassid, and M. Calvin[2]

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As part of a program for the biosynthesis of carbon-labeled biologically important compounds, especially sugars, the following exploratory work has been performed on the assimilation of radioactive carbon dioxide in the light by young barley plants.

Plants were grown in a greenhouse on Hoagland's nutrient solution until they averaged 6–7 inches in height. Upon removal to the laboratory, select plants were divided into two groups: (1) those from which primary and secondary roots and hypocotyl had been cut (hereafter referred to as "minus"); and (2) entire plants, except for secondary roots (hereafter referred to as "plus"). Fresh weights of each group were approximately equal. (The minus group therefore had considerably more leaf material, since the roots and hypocotyls of the plus plants constitute a large fraction of their weight.)

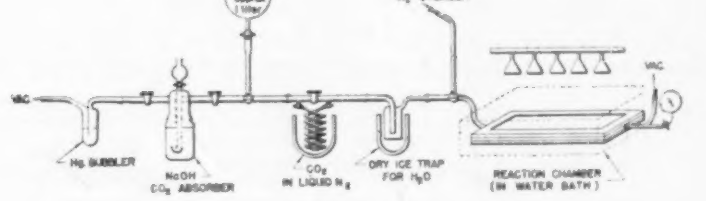

Fig. 1

The reaction chamber housing both groups of plants consisted of a rectangular brass frame sandwiched between two 1-inch glass panes; the free volume was approximately 1 l. The chamber was placed within a tank containing flowing water for cooling (ca. 25°) and illuminated from above, at a distance of 18 inches, with a bank of four GE Projector Spot, 250-watt lamps.

[1] This paper is based on work performed under Contract # W-7405-Eng-48 of the Atomic Energy Commission with the University of California, Berkeley.

[2] With the technical assistance of G. A. Hall.

The setup in the experiment is depicted in Fig. 1. The CO_2 absorber, filled with NaOH, was present to catch any CO_2 escaping the spiral immersed in liquid nitrogen. The helium (or H_2) bulb, containing 2–5 p.s.i. (above atmospheric pressure) of the gas, flushed the spiral containing $C^{14}O_2$ into the chamber. At the beginning and end of the experiment, hydrogen flushed other gases out of the tissue. The dry ice trap was for water.

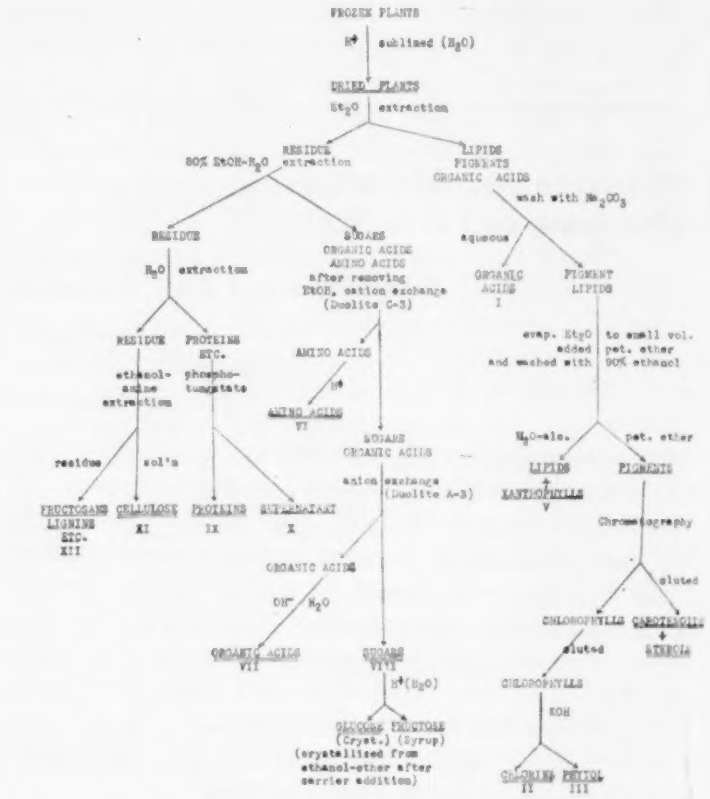

Fig. 2. The substances doubly underlined are materials upon which direct counts were obtained; those singly underlined, materials whose activity was obtained by difference.

TABLE 1

	With roots Total activity fixed (%)	Without roots Total activity fixed (%)
Dried whole plant	100*	100†
Ether extractable acids (I)	[12.5]‡	[7.9]‡
Chlorins (II)		0.8
Phytol (III)		0.9
Carotenoids (IV) (contaminated with sterols)	6.1	3.6
	0.9	
Other lipids (V)		
Amino acids (VI)	7.2	5.6
Acids (VII)	11.3	7.7
Sugars (VIII)	25.8	35.0
Soluble protein (IX)	0.7	6.9
Nonprotein (aqueous) (X)	[3.5]‡	
Cellulose (XI)	2.8	3.6
Lignins, etc. (XII)	[8.3]‡	[9.4]‡
	78.2§	79.7§

* The weight was 2.223 grams—containing 2.00×10^6 c/min.

† The weight was 1.871 grams—containing 0.58×10^6 c/min.

‡ Obtained by calculation rather than direct measurement.

§ Much of the loss is due to incomplete recovery of acids from regenerated adsorption columns.

The chamber was filled with hydrogen and evacuated 5 successive times to remove CO_2, after which the $C^{14}O_2$ (from approximately 45 mg. of $BaC^{14}O_3$) was swept in with the aid of

helium to a vacuum of 23 inches, and finally, hydrogen was admitted to a vacuum of 5 inches. The light was then turned on, and the leaves permitted to photosynthesize for 60 minutes. At the end of the experiment the chamber was slowly evacuated, the remaining CO_2 being caught in the spiral and NaOH bubbler. Six flushings with hydrogen to remove as much CO_2 as possible, the final evacuation being to ca. 29 inches, consumed an additional hour. Th respiration time was therefore two hours.

The plants taken from the chamber were placed immediately into liquid nitrogen and ground to a powder. The frozen powder was then treated as shown in Fig. 2.

The crystalline glucose was obtained by evaporating the hydrolyzed sucrose mixture to a syrup, dissolving this syrup together with several hundred milligrams of carrier glucose in the minimal amount of 95 per cent alcohol, and then adding ether to produce a slight cloudiness. Upon cooling, glucose crystallizes out.

It should be remembered, however, that the above distribution of activity is valid only for the specific set of conditions for photosynthesis given. It may be expected to be different for other conditions, *i.e.* time, O_2 pressure, CO_2 pressure, etc.

The activity measurements of the various fractions shown in the foregoing chart are given in Table 1.

IN THE LABORATORY

A Micromethod for the Determination of 1–(+) Lactic Acid[1]

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Lehmann (*2*) has presented a detailed description of a simple micromethod for determining 1–(+) lactic acid which appears to have been largely overlooked. We have found this basic method very useful and have devised certain modifications which eliminate interfering substances and permit final measurement to be made colorimetrically.

The method depends on the quantitative oxidation of lactic acid to pyruvic acid by means of the yeast enzyme, lactic dehydrogenase, in the presence of potassium ferricyanide, which acts as hydrogen acceptor. Lehmann titrated the resulting potassium ferrocyanide with ceric sulfate, using 1 per cent "setoglaucin-o" as the indicator. Diphenylamine sulfonic acid may likewise be used (*3*) as the indicator. According to Lehmann, the method is quite specific. Of a group of 52 substances investigated, which included carbohydrates, amino acids, α- and β-hydroxybutyric acids, intermediary metabolites, and substances related structurally to lactic acid, the only compounds which interfered with the determination were glycolic, α-hydroxybutyric, and glyceric acids, α-glycerophosphate, hexosediphosphate, and hexosemonophosphate. The first two compounds are not present in sufficient concentration to interfere in biological fluids. The last four can be removed by treatment with $CuSO_4$-$Ca(OH)_2$ according to the method of Friedemann and Kendall (*1*). Ascorbic acid which interferes by reacting directly with potassium ferricyanide can likewise be removed by this procedure. Preliminary treatment with $CuSO_4$-$Ca(OH)_2$ does not interfere with the enzymatic reaction providing the pH is properly adjusted before incubation with the enzyme.

Thus, the procedure used in this laboratory is similar to that described by Lehmann except for the use of $CuSO_4$-$Ca(OH)_2$ for the removal of interfering substances and the determination of potassium ferrocyanide by a colorimetric instead of a titrimetric method. A brief description follows.

Reagents necessary for the method are M/15 Na K phosphate buffer, pH 7.4; 5M/1,000 potassium ferricyanide in water; and ferric salt prepared as follows: Twenty grams of gum ghatti in a cheesecloth bag is soaked in 1 l. of water for 24 hours, and 5 grams of anhydrous $Fe_2(SO_4)_3$ and 75 cc. of 85 per cent H_3PO_4 + 100 cc. of water are added. After mixing, 15 cc. of 1 per cent $KMnO_4$ is added in order to destroy reducing materials in the gum ghatti. The solution is allowed to stand a few days before using.

Fleischmann's baker's yeast is powdered and dried at room temperature. It is then washed several times with distilled water (100 grams in 1 l. of water), following which it again is dried at room temperature and powdered. This dried yeast is the source of the enzyme, lactic dehydrogenase, and keeps indefinitely in the refrigerator. Before using, the yeast is washed once with phosphate buffer (50–100 mg. yeast, depending upon the activity, in 10 cc. of buffer), centrifuged, and resuspended in the same volume of buffer. The activity of the enzyme as well as the removal of intracellular substrate may be tested as suggested by Lehmann by incubating 1-cc. aliquots of the yeast suspension alone, and with 180 γ lactate, in the presence of 1 cc. of a solution of o-chlorophenol-indo-2:6-dichlorophenol (sodium 2,6-dichlorobenzenone-indo-3-chlorophenol (ortho), Eastman P 3467) (1:50,000) in an open test tube. In the presence of lactic acid the indicator should be decolorized in 3 minutes or less. The control should not be decolorized in 10 minutes and usually is not in an hour.

Procedure: The sample containing lactic acid is deproteinized with trichloracetic acid and centrifuged. The supernatant fluid is neutralized and diluted so that 1 cc. contains not more than 200 γ of 1-lactic acid. If ascorbic acid or phosphorylated hexoses be present, the sample is treated with $CuSO_4$-$Ca(OH)_2$ as described by Friedemann and Kendall. Treatment with trichloracetic acid is unnecessary when the $CuSO_4$-$Ca(OH)_2$ precipitation is used in unknowns with small amounts of

[1] This work was supported by a grant from the Mallinckrodt Chemical Works.

proteins. After removal of the $CuSO_4$-$Ca(OH)_2$ precipitate by centrifugation, the sample containing the lactic acid is neutralized. One cc. (containing 200 γ or less) is incubated with 1 cc. of yeast suspension in phosphate buffer and 1 cc. of potassium ferricyanide for ½ hour at 30° in open test tubes. Air does not interfere with the determination. At the end of the incubation period, the mixture is centrifuged. To 2 cc. of the supernatant fluid ferric sulfate solution is added, the mixture is diluted, and the amount of resulting Prussian blue determined using a photoelectric colorimeter with a yellow-green filter. In our experiments the Cenco-Sheard-Sanford photelometer was used. For this instrument we found it best to use two standard curves—one for samples containing less than 75 γ of 1-lactic acid/cc., the other for samples containing 75–150 γ. If the sample contains less than 75 γ/cc., 2 cc. of the supernatant fluid from the enzyme mixture are placed directly in a cuvette, 1 cc. of ferric salt solution and 2 cc. of water are added, and the reading made immediately. For samples containing 75–150 γ, 2 cc. of the supernatant fluid from the enzyme mixture are placed in a 10-cc. volumetric flask, 2 cc. of Fe^{+++} salt added, the mixture diluted to volume, and the Prussian blue determined.

A practically straight-line relationship is obtained when per cent transmission is plotted against concentration. The curve is completely reproducible with the same yeast preparation and when incubated for the same length of time. We found it best, however, to make a standard curve with each set of analyses by setting up one blank determination and one with a known amount of lactic acid, in this way correcting for any variation in the yeast suspension or in the procedure.

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Histochemical Demonstration of Alkaline Phosphatase in Decalcified Dental and Osseous Tissues[1]

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Histochemical studies on alkaline phosphatase in bones and teeth have been restricted to the incipient stage of mineral deposition, due to the fact that it very soon becomes impossible to section these structures without first decalcifying them. Employment of acids to remove the mineral has resulted in destruction of this enzyme. In rats, therefore, it has not been possible to follow the alkaline phosphatase in odontogenesis later than 4 days past birth (*1*).

It is obviously desirable to learn the localization and relative activity of this enzyme in bones and teeth throughout their developmental history. It would be expected that such information might have functional significance. The dental tissues are of extraordinary interest in this regard, since they are highly specialized for mineral deposition.

[1] Aided by a grant from the Milton Fund, Harvard University.

In the development of a method for the successful demineralization of the hard structures without at the same time destroying the alkaline phosphatase, we have been guided by a study of the activity of this enzyme *in vitro* under a variety of environmental and chemical factors (*2*).

The heads of 28-day-old rats were split in the midsagittal plane, fixed in cold, 80 per cent alcohol, and placed in the following solutions of specified pH:

I. (a) equal parts of 20 per cent sodium citrate and 2 per cent formic acid . . . . pH 4.9
   (b) equal parts of 20 per cent sodium citrate and 5 per cent formic acid . . . . pH 4.2

II. (a) 5 per cent aqueous ammonium citrate . . . . pH 4.8
   (b) 10 parts 5 per cent ammonium citrate and 1 part 15 per cent citric acid . . . . pH 4.2

Completion of decalcification, as shown by the needle test, required 1–5 days. During dehydration of the tissue blocks all alcoholic solutions were buffered to pH 9.3 ± 0.1, a step important in preserving the maximum phosphatase activity. In line with this objective it was found necessary to use a minimum of heat to expand the paraffin ribbon; we allowed 10–15 seconds at 45°–50°C. when using paraffin with m.p. 56°–58°C.

The upper incisors and molars with bony encasements in each case were sectioned longitudinally. The sections were uniformly incubated for 3 hours at 37°C., using sodium glycerophosphate as a substrate. Visualization of the enzymatic action was brought about by the generally accepted method of Gomori (*3*), which results in the formation of black cobalt sulfide at the site of enzyme activity.

The range in hydrogen-ion concentration over which decalcification could be accomplished without destroying alkaline phosphatase was found to be very narrow, *i.e.* pH 4.8–5.0. When this range was exceeded on the alkaline side, the rate of decalcification became unduly slow; with a slight increase in acidity the alkaline phosphatase activity was irreversibly lost. This is illustrated by the observation that little or no phosphatase activity was demonstrable in the tissues decalcified in solutions Ib and IIb at pH 4.2. The phosphatase activity was well preserved, however, in comparable tissue blocks decalcified at pH 4.8 and 4.9 (solutions Ia and IIa, respectively). The purpose in buffering all the alcoholic solutions used subsequent to decalcification was to provide immediately an optimum condition for the phosphatase to retain and perhaps to regain activity. That this may be an important factor was further demonstrated by allowing sections to stand 24 hours in an aqueous medium buffered at pH 9.3 ± 0.1 prior to incubation; there was a definite increase in phosphatase activity in comparison with adjacent sections not so treated and which contained demonstrable phosphatase activity. The reaction was also accentuated by the addition of magnesium chloride to the substrate. In fact, when sections were pretreated in the manner just described and subsequently incubated with magnesium ions in the substrate, an amount of precipitate was deposited in the odontogenic and osteogenic tissues which was considered to represent a maximum alkaline phosphatase reaction.

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